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# THE EFFECT OF VARIOUS MANGANESE SOURCES ON EGG QUALITY PARAMETERS OF LAYING HENS

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**Abstract**
This study was conducted to investigate the effect of feed supplementation with different manganese (Mn) sources on performance and egg quality parameters of Lohmann Brown hens. Ninety six 20-weeks-old laying hens were allocated to four dietary treatments. Hens were fed maize-soybean basal diets (BD) which differed only in the form of Mn supplemented. Group 1 were fed unsupplemented BD during the whole experiment. Diets for groups 2, 3 and 4 consisted of BD supplemented with a Mn dose of 30 $mg.kg^{-1}$ in the form of Mn sulphate, Mn chelate of protein hydrolysate (Mn-Pro) and Mn-glycine chelate (Mn-Gly), respectively. During the experiment performance parameters, eggshell qualitative parameters, concentration of manganese and zinc in egg yolks and oxidative stability of egg yolk lipids were determined. During the entire experiment, neither daily feed intake, egg weight nor feed to egg mass ratio were affected by dietary treatments. Egg production was significantly increased in groups supplemented with Mn-Pro and Mn-Gly. Regardless of source, the Mn supplementation to feed significantly increased the relative eggshell weight and eggshell thickness. The proportion of cracked eggs was significantly decreased in groups fed organic sources of Mn. Intake of BD enriched with Mn-sulphate resulted in significantly lower concentration of malondialdehyde in yolk of eggs compared to group fed Mn-Gly. The storage time significantly worsened the oxidative stability of yolk lipids in the group 1, 3 and 4. Our experiment showed that supplementation of hen´s diet with Mn has positive effect on eggshell quality. Diet supplementation with inorganic form could prevent the oxidative damage of yolk lipids during the storage time.

**Introduction**

For the egg producers are very important to increase egg production, egg weight and improve quality of eggshell because these features are related with economic profitability of poultry industry. Eggshell quality is one of the most important egg parameters. Eggs of poor shell quality cause significant losses to producers of consumer eggs. Nonstandard eggs usually account for 3 to 12% of the total yield (Jelínek, 1996; Ledvinka *et al.*, 2000). High breaking strength of eggshell and absence of shell defects are essential for protection against the penetration of pathogenic bacteria such as *Salmonella sp.* into eggs (Swiatkiewicz *et al.*, 2010). Eggshell quality is most often expressed as the proportion of the eggshell in egg weight, and by thickness. It is well known that main biological factors affecting eggshell quality are internal factors such as genetics, hen's age, production cycle stage, egg weight, intensity and persistence of production, time of laying, combined with external factors such as the nutritional level, farm system and microclimatic parameters. From nutritional point of view is the quality of eggshell often connected to macrominerals (Ca, P) and vitamin D3 influence, but nowadays it is well known that trace elements are also very important in mineralization process. Manganese and zinc, as cofactors of metaloenzymes responsible for carbonate and mucopolysaccharides synthesis, play an important role in eggshell formation (Swiatkiewicz and Koreleski, 2008). Mabe *et al.* (2003) suggested that trace elements as Mn, Zn and Cu could

affect mechanical properties of eggshell by effect on calcite crystal formation and modifying crystallographic structure of eggshell. Mn may affect eggshell quality, because activates the glycosyl transferases involved in the formation of mucopolysaccharides, which are components of proteoglycans (Leach, 1976). Mn also playes crucial role in antioxidant protection, because it is integral part of Mn-superoxide dismutase (Mn-SOD) (Underwood and Suttle, 1999).

In recent years, studies on the efficacy of organic trace minerals in animals have received increasing attention. Compared with inorganic minerals, organic minerals have several benefits including: protect from unwanted chemical reaction in gastrointestinal tract; easily pass intact through intestine wall; and be absorbed by different routes (Mateos *et al.*, 2005). Regarding the use of organic sources the results of the studies are still inconsistent. Some studies suggested that use of organic sources of Mn substantially affects laying performance and eggshell quality (Yildiz *et al.*, 2011; Sun *et al.*, 2012), whereas others authors claimed that there is no difference between inorganic and organic sources of Mn (Lim and Paik, 2003; Mabe *et al.*, 2003; Swiatkiewicz and Koreleski, 2008).

The objective of this study was to compare the efficacy of dietary organic Mn sources and $MnSO_4.H_2O$ by investigation their effects on egg quality parameters, laying performance, lipid oxidation of egg yolks during storage and mineral retention in egg yolks of laying hens.

**Material and methods**

A total of 96 hens of laying strain Lohmann Brown, 20 weeks old were randomly allotted into 4 equal groups according to the dietary treatment. Each group consisted of 6 replicates with 4 hens in each. For three weeks of adaptation period, all experimental groups were fed with a basal diet (BD) based on maize and soybean meal. During next 8 weeks the birds were fed the same BD which differed only in the form of Mn supplemented. Group 1 continued on unsupplemented BD (analyzed Mn content 52 $mg.kg^{-1}$ DM). Diets for groups 2, 3 and 4 consisted of BD supplemented with a Mn dose of 30 $mg.kg^{-1}$ in the form of Mn sulphate, Mn chelate of protein hydrolysate (Mn-Pro) and Mn-glycine chelate (Mn-Gly), respectively. The experiment lasted for 11 weeks. During the whole experiment the layers were housed in room with a controlled ventilation and lighting (15hours/day) at 2 hens per cage. During experiment hens had constant access to water and feed. All experimental procedures were in accordance with established standards for the care and use of animals for research purposes.

Birds were individually weighed at the beginning and at the end of the experiment. Egg production, egg weight, number of cracked eggs were recorded daily during the 8 weeks of experimental period. Feed intake (per cage) was registered weekly. Using collected data, basic production parameters (egg mass, feed to egg mass ratio, daily feed intake) were calculated. At $31^{th}$ week of age three eggs from each replicate (18 eggs/treatment/day), for two consecutive days were collected to determine the egg quality parameters (eggshell weight (g), eggshell thickness (mm), eggshell proportion (%), yolk and albumen weight (g), yolk and albumen proportion (%), eggshape index (%)). Eggs were broken, and the albumens and yolks were separated and weighed. Eggshell weight was measured after washing the interior egg membrane and after drying for 48 hours at 60°C. Eggshell thickness was measured using micrometer (Model 7313, Mitutoyo corp., Japan) as the average of both ends (air cell and sharp end) and at the middle, without the shell membranes. The proportion of eggshell (ES), albumen (A) and yolk (Y) were calculated ((ES or A or Y weight/egg weight)x100). The short and long diameters of the eggs were measured with a micrometer to determine egg shape index.

At the end of experimental period 3 eggs per replicate were collected and yolks were pooled for analysis of Mn and Cu concentration by flame atomic absorption spectrophotometry using AAS Shimadzu (Kyoto, Japan) Model AA-700.

The malondialdehyde concentration in the yolks (3 eggs per replicate, total 18 eggs/treatment) of fresh eggs and eggs stored for 10, 20, 30, 40 days at 4°C was measured by the modified fluorometric method in accordance with Jo and Ahn (1998).

Statistical analysis of the differences between the groups was carried out using one-way analysis of variance with the post hoc Tukey multiple comparison test. Differences were considered significant at $P<0.05$. All statistical analysis were performed with GraphPad Prism 5.0 software.

**Results and discussion**

The effects of dietary Mn supplementation on laying performance parameters are presented in Table 1. The Mn supplementation source has not statistically significant effect on the weight gain, feed intake, egg weight and on the feed to egg mass ratio. By contrast, the egg production was significantly increased in laying hens supplemented with organic sources of Mn. Yildiz *et al.* (2011) reported that body weight gains and the egg weights were significantly enhanced when Mn was used as organic form (Mn-Pro). On the contrary Swiatkiewicz and Koreleski (2008) observed no beneficial effects of organic Mn sources on the laying performance parameters. The National Research Council (1994) estimated the requirement for manganese as 20 $mg.kg^{-1}$ per hen per day. Maurice and Whisenhut (1980) , and Sazzad *et al.* (1994) reported no significant differences in egg production, egg weight and feed conversion with increasing manganese level in the diet. Similar results observed also Luo *et al.* (2003) who reported no effect of supplemental Mn (30-120 $mg.kg^{-1}$) on egg production in brown layers. However, in this study, hen-day egg production was affected by supplementing 30 $mg.kg^{-1}$ Mn with both organic forms. The basal diet containing 52 $mg.kg^{-1}$ Mn provided sufficient amount for the other laying performance parameters.

*Table 1*

**Treatment effects on performance of laying hens**

| Item | Treatment | | | |
|---|---|---|---|---|
| | Basal diet (BD) | BD + Mn sulphate | BD + Mn-Pro | BD + Mn-Gly |
| Weight gain 20-31 wk (g) | 186.5 ± 18.31 | 208.6 ± 43.6 | 153.9 ± 11.93 | 151,0 ± 41.91 |
| Feed intake (g/hen/day) | 114.2 ± 1.13 | 116.3 ± 0.98 | 114.3 ± 0.97 | 113.6 ± 1.13 |
| Egg production (egg/hen/day) | $0.97 \pm 0.005^{a}$ | $0.98 \pm 0.003^{ab}$ | $0.99 \pm 0.004^{b}$ | $0.99 \pm 0.003^{b}$ |
| Egg weight (g/egg) | 59.20 ± 0.41 | 58.32 ± 0.47 | 58.62 ± 0.57 | 59.77 ± 0.49 |
| Feed to egg mass ratio (g feed/g egg) | 2.00 ± 0.03 | 2.04 ± 0.02 | 1.98 ± 0.03 | 1.92 ± 0.03 |

$^{a,b}$means in the same row with different superscripts differ significantly ($P \leq 0.05$)

Trace elements may affect eggshell quality by their catalytic properties as key enzymes involved in the process of membrane and formation of eggshells or by interaction directly with the calcite crystals in the formation of eggshells (Mabe *et al.*, 2003). The increased eggshell thickness in our experiment could be the effect of higher Mn dose in the diets compared to control group. In this study, all investigated parameters of the eggshell quality were significantly influenced by Mn intake (Table 2). The eggshell weight and thickness have been significantly elevated in all supplemented groups, regardless of the Mn source. Similar results were found by Mabe *et al.* (2003) who reported no differences in eggshell proportion and eggshell density between hens fed diet supplemented with inorganic and organic sources of

Mn, Zn and Cu. In contrary Bunesova (1999) and Klecker *et al.* (2002) found positive effect of partial substitution of inorganic Mn and Zn sources with their organic forms on eggshell weight and eggshell thickness. The proportions of the cracked eggs has appeared to be reduced in laying hens receiving the organic Mn sources than in those fed unsupplemented diet ($P < 0.05$). Similar results were found by Yildiz *et al.* (2011). The eggshell proportion was significantly increased in groups supplemented with Mn sulphate and Mn-proteinate.

*Table 2*

**Effects of dietary manganese supplementation on parameters of egg quality**

| Item | Treatment | | | |
|---|---|---|---|---|
| | Basal diet (BD) | BD + Mn sulphate | BD + Mn-Pro | BD + Mn-Gly |
| Egg weight (g) | 60.54 ± 0.91 | 59.32 ± 1.07 | 61.51 ± 1.36 | 61.01 ± 0.43 |
| Albumen weight (g) | 40.31 ± 0.98 | 39.06 ± 0.94 | 40.51 ± 1.11 | 40.19 ± 0.51 |
| Yolk weight (g) | 14.79 ± 0.16 | 14.39 ± 0.08 | 14.99 ± 0.21 | 15.02 ± 0.20 |
| Eggshell weight (g) | $5.45 \pm 0.06^{a}$ | $5.89 \pm 0.13^{b}$ | $6.02 \pm 0.12^{b}$ | $5.86 \pm 0.05^{b}$ |
| Albumen proportion (%) | 66.54 ± 0.65 | 65.80 ± 0.37 | 65.81 ± 0.39 | 65.85 ± 0.43 |
| Yolk proportion (%) | 24.5 ± 0.49 | 24.3 ± 0.35 | 24.4 ± 0.27 | 24.6 ± 0.42 |
| Eggshell proportion (%) | $9.01 \pm 0.16^{a}$ | $9.93 \pm 0.15^{b}$ | $9.79 \pm 0.17^{b}$ | $9.61 \pm 0.12^{ab}$ |
| Eggshell thickness (mm) | $0.36 \pm 0.004^{a}$ | $0.38 \pm 0.003^{b}$ | $0.39 \pm 0.006^{b}$ | $0.38 \pm 0.003^{b}$ |
| Egg shape index (%) | 78.4 ± 0.53 | 77.7 ± 0.56 | 77.9 ± 0.50 | 78.6 ± 0.15 |
| Cracked eggs (%) | $3.63 \pm 0.66^{a}$ | $2.31 \pm 0.46^{ab}$ | $1.35 \pm 0.33^{b}$ | $0.78 \pm 0.30^{b}$ |

$^{a,b}$means in the same row with different superscripts differ significantly ($P \leq 0.05$)

The malondialdehyde (MDA) values of egg yolks, as a marker of lipid peroxidation are presented in Table 3. A moderate increase in the concentration of products of lipid peroxidation measured as MDA in the yolk was observed in all experimental groups during the refrigerated storage of eggs. The MDA values were almost equivalent in all treated groups, although after 40$^{th}$ day of storage the lipid peroxidation in yolk of eggs in group 4 was significantly increased compared to the group supplemented with inorganic form. The information about the dietary Mn supplementation on the oxidative stability of egg yolk lipids are insufficient. We can only suppose that lipid peroxidation in egg yolk may be affected by altered activity of superoxide dismutase. Wawrzykowski and Kankofer (2011) found that the activity of superoxide dismutase in egg yolk did not change during 6 days storage but between 6$^{th}$ and 9$^{th}$ day, it decreased significantly.

*Table 3*

**Malondialdehyde concentration in egg yolks during storage periods (mg/kg yolk)**

| Storage period | Treatment | | | |
|---|---|---|---|---|
| | Basal diet (BD) | BD + Mn sulphate | BD + Mn-Pro | BD + Mn-Gly |
| 0$^{th}$ day | $0.70 \pm 0.05^{a}$ | 0.83 ± 0.06 | $0.76 \pm 0.06^{a}$ | $0.78 \pm 0.04^{a}$ |
| 10$^{th}$ day | $0.82 \pm 0.04^{ac}$ | 0.99 ± 0.03 | $0.88 \pm 0.05^{ab}$ | $0.95 \pm 0.05^{ab}$ |
| 20$^{th}$ day | $1.07 \pm 0.09^{b}$ | 1.01 ± 0.13 | $1.05 \pm 0.06^{b}$ | $0.88 \pm 0.06^{a}$ |
| 30$^{th}$ day | $0.95 \pm 0.04^{bc}$ | 1.06 ± 0.02 | $1.08 \pm 0.08^{b}$ | $1.10 \pm 0.06^{b}$ |
| 40$^{th}$ day | $1.03 \pm 0.03^{bcAB}$ | $0.96 \pm 0.02^{A}$ | $1.03 \pm 0.06^{bAB}$ | $1.13 \pm 0.03^{bB}$ |

$^{a,b}$means in the same column with different superscripts differ significantly ($P \leq 0.05$)

$^{A,B}$means in the same row with different superscripts differ significantly ($P \leq 0.05$)

In our experiment the addition of various forms of manganese at the dose of 30 mg/kg into diet did not significantly influenced the deposition of Mn and Zn in egg yolks .

*Table 4*

**Treatment effects on mineral concentration in egg yolks (mg/kg DM)**

| Item | Treatment | | | |
|---|---|---|---|---|
| | Basal diet (BD) | BD + Mn sulphate | BD + Mn-Pro | BD + Mn-Gly |
| Manganese | 1.65 ± 0.16 | 1.77 ± 0.08 | 1.66 ± 0.12 | 1.73 ± 0.16 |
| Zinc | 78.6 ± 2.73 | 84.4 ± 1.67 | 83.5 ± 3.69 | 80.3 ± 1.93 |

**Conclusion**

Based on this study we concluded that the Mn supplementation with different forms (organic vs. inorganic) in the diet for laying hens positively affect quality of eggshell. Intake of BD with organic Mn sources positively affect the egg production and decreased the proportion of cracked eggs. Diet supplementation with inorganic form could reduce the negative effect of oxidative damage of yolk lipids during the storage time.

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